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Estimating age using permanent molars and third cervical vertebrae shape with a novel semi-automated method

Scheila Mânica¹, Ferranti SL Wong¹, Graham Davis¹, Helen M Liversidge¹

¹PhD, Institute of Dentistry, Queen Mary University of London, UK.

Corresponding author: scheila.manica@qmul.ac.uk

The authors declare that they have no conflict of interest

Highlights section:

- Third cervical vertebral shape can discern between individuals age <18 and ≥18 years
- Useful in cases when the second molar is mature or the third molar is missing
- Reduces the impact of highly variable third molar development in age estimation

ABSTRACT: Estimating chronological age accurately in young adults is difficult and additional methods are required to increase the accuracy. This study explored a new semi-automated method to assess shape change of third cervical vertebra (C3) with age in the living; comparing this as a method to determine whether individuals could be categorised into being less than 18 years of age (<18), or at least 18 years of age (≥18) with tooth formation of the second and third mandibular molars (M2 and M3). The sample was panoramic and lateral skull radiographs of 174 dental patients (78 males, 96 females aged 15-22 years). Twelve variables were compared in two age categories: younger than 18 and at least 18 years of age in males and females separately using a t-test. Tooth formation of M2 and M3 was assessed. Mean values of eight variables of C3 in males and one variable in females were significantly different between the two age categories ($p < 0.05$). Results for males showed that the best age indicator for age ≥18 was the ratio between height and width of C3 and for females, the ratio between diagonals. Results for molars showed that M2 was mature in 69% of males and 83% of females, within the expected age range of 14-16 years. M3 was highly variable ranging from stages 6 to 14 for both; M3 was missing in 24% of males and 28% of females and mature in 14% of males and 15% of females. The conclusion was that shape change of C3 has potential as an additional method to group individuals <18 and ≥18 years of age.

KEYWORDS: Forensic science, third cervical vertebrae, molar, maturation, legal age

INTRODUCTION

Metric (e.g: body height) and non-metric (e.g: assessment of dental development) methods of age estimation are used in skeletal remains in order to estimate age-at-death and to form a biological profile.(1) In living individuals, the assessment of age is required in children and young adults with no documents for immigration control or asylum-seeking process; (2) and to determine whether the person concerned has reached the age of criminal responsibility or not.(3) It is claimed that the complexity of the study on human development is attenuated when different markers used for age estimation are combined. Variations in human development are better understood

when the relationship between dental, skeletal and chronological age in children is analysed.(4) However, the difficulty of age assessment increases with age as individual teeth/bones reach maturity. After the second mandibular molar (M2) reaches maturity (on average around 14 years), the only remaining developing tooth available to estimate age is the third molar (M3). If M3 is mature, then the clavicle or other bones must be used to estimate age.

A range of techniques to assess human growth and development exist, but simple, non-invasive, and low-cost methods are preferable. Skull radiographs are frequently taken for orthodontic treatment and other medical purposes, such as for the diagnosis of pathological calcifications in the cervical region, (5) as well as for age estimation in living person. (6) For instance, lateral cephalogram (LC) of the skull assists with the visualization of lateral craniofacial skeleton, cervical vertebrae maturation (CVM) and dental development. Skeletal analysis compare linear and angular measurements from landmarks that change during growth.(7) Dental development is monitored through a sequence of events from initial mineralization, crown formation, root growth and root apex maturation.(8) The third cervical vertebrae have a trapezoidal shape and appear like a wedge of cheese where the posterior vertical height is longer than the anterior vertical height.(9) The shape changes from a wedge to a rectangle and then to a square during growth between ages 10-15 years. This anatomical change comes about by superior and inferior length increments being less than anterior and posterior height increments(10).

The aim of this study was to assess the potential of CVM growth changes during late adolescence and early adulthood. This was done by analysing maturation of different radiographic markers of a group of Southern Brazilian dental patients aged 15 – 22 years. The markers chosen were the third cervical vertebrae (C3) and the second (M2) and third molar (M3) on the left side of the mandible.

MATERIALS AND METHODS

The data from this cross-sectional study was obtained from anonymised archived lateral cephalogram (LC) and panoramic (OPG) radiographs. Those were taken with consent as part of routine orthodontic records of two Brazilian private radiographic clinics based in *Florianópolis* (capital city of Santa Catarina, South of Brazil). The sample included 174 Southern Brazilian adolescents and young adults of mixed ethnicity - 78 males and 96 females, aged 15 to 22 years - selected from 2013 to 2014 (Table 1). The inclusion criteria were: (a) subjects with no history of facial trauma and absence of congenital malformations, (b) both radiograph taken on the same day and (c) good visibility of C3 and M2/M3 lower left side in both radiographs.

Table 1

Cervical vertebra maturation

The images were uploaded in an open access image processing program, *ImageJ*, 1.47v(11) and the following steps were used to generate a geometric outline of the C3 vertebra. Firstly, five points (dots) were equally placed along the border in each of the four corners of cervical vertebrae with the middle point on the apex; three points (rectangle dots) were placed on the lower border in equal distance from the inner black dots ($1/4$; $1/2$; $3/4$); and one point was placed at the middle ($1/2$) between the apex points on the other three borders (shown in Figure 1 and 2). The outline of the C3 body was drawn using *ImageJ*, starting from top left in a clockwise direction. The

corresponding Cartesian co-ordinates for each image were recorded for further investigation and analysis.

Figure 1. Lateral cephalogram with C3 outline drawn using image J.

Figure 2. Identification of landmarks on the corners and edges of the outline of the third cervical vertebra after standardising the inferior border on the horizontal.

An in-house program called '*quadfit*' was used to align the cartesian co-ordinates to the vertebrae outline and enable the following automatic geometric measurements of the C3 (shown in Figure 3): two diagonals K2 and K3 (1 and 2) formed from two extreme points (d1, e1) and (d3, e3) for K2 (3 and 4) and (d2, e2) and (d4, e4) for K3 (5 and 6). The length of K2 and K3 were divided in two, each one, by the centre of intersection and named K2.1 and K2.2 (7 and 8) and K3.1 and K3.2 (9 and 10), respectively. The width of C3 was the distance between the points (d1, e1) and (d2, e2) and the height of C3 were calculated from the perpendicular distance from the width. It was noted that the two centres of the cervical body: the intersection of the two diagonals (dot, 11) and the centre of the mass was defined as a mathematical centre for an irregular shape (cross, 12), were not always coincident and the distance between these two centres was calculated. Finally, the total area was calculated automatically and a parallelogram created by the connection of the four points (d1,e1/d2,e2/d3,e3/d4,e4) guided the calculation of the area of the lower border, which is concave and hence a negative value.

Figure 3. Identification of dimensions assessing shape changes of the third cervical vertebra. See text for explanation of measurements.

Dental maturation

The table of Moorrees et al.(12) modified by Liversidge(13) (Table 4 2) was used to assess the dental maturation stages of the lower left second (M2) and third (M3) molars in LC. As shown on table 2 each stage was numbered from 1-15 relating to the corresponding stage of crown or root development for the tooth.(14) If the root apex of M2 or M3 was mature, age was not estimated. Both types of radiographs were assessed on the left side. The left side of the mandible on the LC was distinguished by being the most anterior and superior part due to the proximity to the radiographic chassis.

Table 2

Statistical analysis

The software SPSS Statistics v19 was used to analyse all the results. Reliability of the accuracy of the measurements of C3 was assessed by a paired t-test for both intra and inter-observer errors in 18 individuals. Kappa intra-observer agreement was performed for the molar stages.

Independent t-tests were performed for the mean value of the measurements of C3 for individuals grouped into less than 18 (<18) and at least 18 (≥ 18). The measure parameters for C3 were categorised into two age groups, <18 or ≥ 18 years old. Independent t-tests were applied to test whether there were significant differences between the two group for each parameter.

Tooth stage assessment of M2 and M3 was compared between lateral cephalogram and panoramic radiographs by the first author. Kappa coefficient was used to compare the readings of M2 and M3 maturation for both types of radiographs.

The mean difference and absolute difference of the age estimations of the M2 and M3 readings of LC were calculated and compared to the chronological ages using a paired t-test.

RESULTS

Reliability

This new semi-automated method assessing C3 shape was validated by reliability results showing no statistically significant difference for both inter- and intra- observer of all measurements ($p>0.05$) for 18 individuals. Results for reliability of tooth stage assessment showed a substantial agreement (0.61-0.80) for the readings of M2 between the types of radiographs and perfect agreement (0.81 – 0.99) for the readings of M3 from the panoramic, M2 from the lateral cephalogram and M3 from the lateral cephalogram.

C3 shape change and age categories

Results are shown in Table 2, eight C3 measurements had significant differences between <18 or ≥ 18 years old groups for males and the best predictor was the ratio between height and width of C3 whilst for females just one measurement, the ratio between diagonals as shown in the table 3.

Age estimation M2 and M3

The M2 was included in this study in order to investigate if maturation was complete by age 15.

The results for M2 showed that 24 of the 78 males and 17 of 96 females had developing M2's and age could be estimated from root stages of M2. Root stages were 13 and 14 (males) and 12 to 14 (females). The mean difference between estimated and chronological ages in males was -1.40 year (SD=0.475, SE=0.097) and absolute mean difference of 1.40 year. Results for M2 in females were a mean difference of -0.870 year (SD=0.663, SE=0.161) and absolute mean difference of 1.870 year. Age was underestimated for all males and females from M2. The 41 readings for M2 were pooled between males and females and, according to the paired t-test, the mean difference was of -1.595 and the absolute mean difference was of 1.595 (SD=0.601/SE=.093).

The results for M3 showed that 48 males and 54 females had developing M3's and age could be estimated from root stages of M3. Root stages were 6 to 14 and the mean difference between estimated and chronological ages was -0.861 year (SD=1.554, SE=0.224) and absolute mean difference of 0.861 year. Results for females were mean difference of -1.116 year (SD=1.423, SE=0.193) and absolute mean difference of 1.116 year. Age was overestimated for 14 males and underestimated for 34 males. Age was overestimated for 12 females and underestimated for 42 females. The 102 readings for M3 of males and females were pooled and the mean difference was of -0.996 (SD=1.485/SE=.147) and the absolute mean difference was of .996.

The M2 was not present in 1 female and M3 was not present in 19 males and 27 females. Although dental history (including previous extraction history) of these patients was unavailable, it is likely that some of the individuals presented with hypodontia, particularly those of younger ages.

Tables 3 and 4

DISCUSSION

Our main findings was that the mean values of eight variables in males and one variable in females of shape assessment of C3 were significantly different between individuals < 18 and ≥ 18 years of age. This suggests that assessing shape changes of C3 is a potential additional method to assess age. The threshold value to be used depends on a number of factors because sensibility and specificity have an inverse relationship. Further studies are needed to optimise and discriminate the threshold values that are acceptable in court if those variables are to be used as part of assessment for estimating age. We also found more parameters of C3 measurement in males than females that could be used to discriminate individuals at 18 years old. This may be due to females having an earlier maturation phase in their cervical vertebrates and only small changes between adolescence and the young adult periods.

The major difficulty of the analysis was the manual tracing of the body of C3 and the challenges were: (1) the visualisation of the corners of C3 which were not always well defined, mostly the corner '3'; and (2) the superposition of other bony structures making identification of landmarks difficult. This was overcome by digitising a number of featured points and using an automated method to produce an outline for measurements. Using this approach, both intra- and inter-observer's agreements of the eight variables were excellent. For manual measurement, ~~it was shown that the~~ mean intra- and inter-observer variation is lower for experienced readers than for radiology residents suggesting that the reliability of bone age measurements increases with experience. (15) The use of an automated outline method in the method developed for this study, can reduce the operator's variation and is independent of experience.

The C3 measurements were carried out in a cross-sectional sample that, comparing to longitudinal study, is relatively insensitive to the patient variability. (16) Other factors that might have influenced our findings that were not known include stature, weight and body mass index, diet and history of geographic location during biological growth and development. Moreover, the sample of Southern Brazilians were of mixed ethnic origin race and this should be taken into consideration when comparing these results with other studies.

The results from this study on accuracy of age estimation from M2 and M3 are fairly good due to the limited age range of this study sample. This is in agreement with a study in Sweden which also assessed age using the third molar development. Their results showed a very low accuracy with a systematic underestimation of age, increasing with age in males and females. The mean difference between chronological age and third molar maturation was ± 0.8 years. (17) A study within the Hispanic population found that the mean absolute difference between chronological age and estimated age was ± 3.0 years in females and ± 2.6 years in males. (18) Another study in Spaniards showed a mean difference between chronological and estimated age of

-0.10 years (± 1.23 SD) for left third molar with slight variations regarding sex. (19) Factors that influence results of accuracy include the age range of the sample, the uneven age distribution, history of agenesis and previous extraction.

Age estimation

The use of multiple skeletal regions to estimate age is considered to be more accurate than using just one single indicator (20). Reliability of methods of age estimation is fundamental and the professional must keep up-to-date with the developments in existent methodologies (21) and the creation of new ones. A recent study showed that most variation in bone age indicators are associated with other factors other than age, such as: hormonal factors, energy balance, biomechanical variables and genetic factors.(22) Any information such as medical history, drug abuse and lifestyle should also be considered.(21)

Suggested requirements for a valid age indicator are the following: 1) the characteristic employed displays progressive and unidirectional change with age; 2) it should be possible to categorise and measure the morphological changes and the results must have low observer error; 3) the morphological changes should occur roughly at the same time in all people with possible divisions for sex and ancestry (23); 4) variability of maturation within the individual must be considered; 5) there is a clear sexual dimorphism within human growth and maturation as females tend to be advanced relative to males.(24)

The major strength of the methodology developed in this study is the automated nature of assessing shape changes of C3. The assessment of cervical vertebrae maturation has previously been largely subjective. The methodology developed in this study relies on an initial subjective outlining the shape of C3. Once this is done, the programme automatically calculates the dimensions and ratios, removing the subjective nature of assessing shape. Automated systems of age assessments are in growing demand because they speed up the huge volume process of human identification, save money and have better precision and accuracy. (25) This is new method, hence, there is no large scale validation but our findings suggest that this approach has important potential.

Our study applies a novel approach to quantify the change in shape of C3 with growth, and builds on previous work assessing growth changes in C3. Documented methods of cervical vertebrae maturation used in orthodontics have been developed to identify the pubertal growth spurt. The 'mature' shape can vary considerably in adults. (26) Our new approach includes the measurement of diagonals and the calculation of the angle in the centre that have not previously been used to quantify growth. This research shows that small morphological changes in shape can be measured, however, these measurements do not always change in a regular way relative to chronological age. It is likely that shape changes of vertebrae continue throughout life(27), and there is a need for new methods assessing such changes in other groups as well as from longitudinal radiographs. The aim to find distinctive patterns in the cervical vertebrae maturation related to legal ages was not fulfilled. However, this study shows how difficult it is to measure biological variation of C3 shape and relate this to chronological age.

CONCLUSION

In conclusion, the semi-automated method developed in this study measuring the changing shape of the third cervical vertebra has potential to group individuals < 18 and \geq 18 years of age. This new method of estimating age from C3 is of particular importance and value if M3 is missing or mature in young adults.

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Table 1. Age and sex distribution of radiographic sample. Age in years. 15 includes age 15.00 to 15.99 etc.

Table 1– Age and sex distribution of radiographic sample.			
Age (years)	Males	Females	Pooled
15	23	14	37
16	6	14	20
17	6	11	17
18	5	14	19
19	6	9	15
20	15	11	26
21	9	12	21
22	8	11	19
	TOTAL = 78	TOTAL = 96	
TOTAL = 174			

Table 2. Crown and root formation stages for the mandibular second molar (M2) and third molar (M3) after Liversidge, (2009). Mean age in years.

Table 2 – Crown and root formation stages for the mandibular second molar (M2) and third molar (M3) after Liversidge, (2009). Mean age in years.			
Code for stages	Description of stages	Mean age M2	Mean age M3
Cr – 1	crypt	2.89	9.16
Ci – 2	cuspid tip initiation	3.68	9.92
Cco – 3	coalescence of cuspid tips	4.52	10.62
Coc – 4	cuspid outline complete	5.26	11.33
C _{1/2} – 5	crown ½ fractions	6.02	12.34
C _{3/4} – 6	crown ¾ fraction	6.97	13.49
Cc – 7	crown complete	7.74	14.28
Ri – 8	initial root	8.33	14.78
Rcl – 9	root cleft formation	8.94	15.19
R _{1/4} – 10	root ¼ fraction	9.88	16.10
R _{1/2} – 11	root ½ fraction	11.14	17.12
R _{3/4} – 12	root ¾ fraction	12.33	17.94
Rc – 13	root length complete	13.24	18.74
A _{1/2} – 14	apex half closed	14.08	19.68
Ac – 15	apex closed	No age estimation	No age estimation

Table 3. Results of third cervical vertebrae (C3) measurements in males for age categories younger than 18 years (<18) and at least 18 years if age (≥ 18). For descriptors of measurements, see Figure 3. Mean values in pixels. SD standard deviation, SE standard error, shaded blocks significance indicate significant or highly significant difference between age categories.

Table 3 – Results of third cervical vertebrae (C3) measurements in males for age categories younger than 18 years (<18) and at least 18 years if age (≥ 18). For descriptors of measurements, see Figure 3. Mean values in pixels. SD standard deviation, SE standard error, shaded blocks significance indicate significant or highly significant difference between age categories.					
Measurements	Age18*	Mean	SD	SE	Significant (2-tailed)
Ratio of height and width	<18	1.67	0.41	0.07	<0.0001
	≥ 18	1.35	0.32	0.049	
Length13 (K2), formed from two extreme points (d1, e1) and (d3, e3)	<18	21.03	1.78	0.30	<0.0001
	≥ 18	22.77	1.70	0.25	
Length24 (K3), formed from two extreme points (d2, e2) and (d4, e4)	<18	16.35	1.21	0.20	<0.0001
	≥ 18	17.78	1.48	0.22	
Length13.length24 (ratio of K2/K3)	<18	0.77	0.033	0.00	0.758
	≥ 18	0.78	0.031	0.00	
Ratio13 (K2.1/K2.2) Ratio of the two parts of K2	<18	1.02	0.13	0.02	0.137
	≥ 18	0.98	0.10	0.01	
Ratio24 (K3.1/K3.2) Ratio of the two parts of K3	<18	1.23	0.20	0.03	0.044
	≥ 18	1.15	0.13	0.02	
Area % lower curvature	<18	-.73	1.68	0.28	0.035
	≥ 18	-1.53	1.52	0.23	
Top angle	<18	86.24	10.79	1.82	0.002
	≥ 18	93.08	6.39	0.97	
Distance between centres	<18	0.85	0.53	0.09	0.274
	≥ 18	0.73	0.40	0.06	
Width of lower curvature	<18	424.14	37.26	6.29	0.153
	≥ 18	437.42	44.01	6.71	

Height of lower curvature	<18	51.20	17.04	2.88	<0.0001
	≥18	66.02	16.27	2.48	
Ratio of height and width of lower curvature	<18	8.98	3.13	0.53	0.002
	≥18	7.01	1.83	0.27	

Table 4. Results of third cervical vertebrae (C3) measurements in females for age categories younger than 18 years (<18) and at least 18 years if age (≥ 18). For descriptors of measurements, see Figure 3. Mean values in pixels. SD standard deviation, SE standard error, shaded blocks significance indicate significant or highly significant difference between age categories.

Table 4. Results of third cervical vertebrae (C3) measurements in females for age categories younger than 18 years (<18) and at least 18 years if age (≥ 18). For descriptors of measurements, see Figure 3. Mean values in pixels. SD standard deviation, SE standard error, shaded blocks significance indicate significant or highly significant difference between age categories.					
Measurements	Age18	Mean	SD	SE	Significant (2-tailed)
Ratio of height and width	<18	1.66	0.42	0.06	1.56
	≥ 18	1.56	0.30	0.039	
Length13 (K2), formed from two extreme points (d1, e1) and (d3, e3)	<18	20.30	1.24	0.20	0.522
	≥ 18	20.13	1.25	0.16	
Length24 (K3), formed from two extreme points (d2, e2) and (d4, e4)	<18	15.44	0.96	0.15	0.086
	≥ 18	15.80	1.04	0.13	
Length13.length24 (ratio of K2/K3)	<18	0.76	0.03	0.00	0.004
	≥ 18	0.78	0.03	0.00	
Ratio13 (K2.1/K2.2) Ratio of the two parts of K2	<18	1.02	0.12	0.02	0.268
	≥ 18	0.99	0.08	0.01	
Ratio24 (K3.1/K3.2) Ratio of the two parts of K3	<18	1.18	0.11	0.018	0.384
	≥ 18	1.16	0.09	0.012	
Area % lower curvature	<18	-2.41	2.25	0.36	0.666
	≥ 18	-2.24	1.55	0.20	
Top angle	<18	93.25	6.49	1.05	0.069
	≥ 18	90.76	6.45	0.84	
Distance between centres	<18	0.78	0.56	0.09	0.161
	≥ 18	0.64	0.40	0.05	
Width of lower curvature	<18	383.37	31.16	5.05	0.183

	≥ 18	393.36	38.39	5.04	
Height of lower curvature	< 18	58.95	10.94	1.77	0.922
	≥ 18	59.19	12.39	1.62	
Ratio of height and width of lower curvature	< 18	6.75	1.54	0.25	0.545
	≥ 18	6.97	1.77	0.23	

Figure 1. Lateral cephalogram with C3 outline drawn using image J.

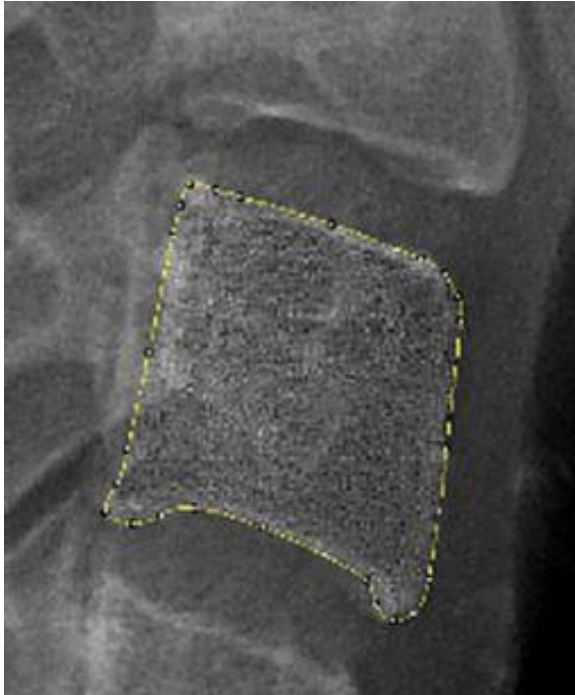


Figure 2. Identification of landmarks on the corners and edges of the outline of the third cervical vertebra after standardising the inferior border on the horizontal.

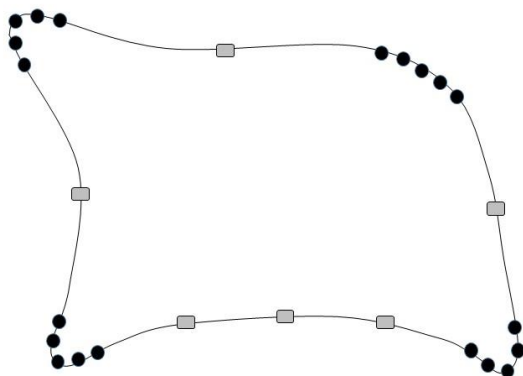


Figure 3. Identification of dimensions assessing shape changes of the third cervical vertebra. See text for explanation of measurements.

